

Appl. No. : Unassigned
Filed : Herewith

AMENDMENTS TO THE CLAIMS

1. (Currently amended) A method of amplifying, and optionally also detecting, a target nucleic acid sequence, the method comprising the steps of:

a) providing a sample that may or may not comprise a target nucleic acid sequence,

b) providing a pair of outer primers and a pair of inner primer, a nucleic acid polymerase and standard reagents for PCR, the melting temperature (T_m) of the pair of outer primers being at least 2 °C higher than the T_m of the pair of inner primers,

c) contacting the sample with the pair of outer primer and the pair of inner primers, and standard reagents for PCR, thus obtaining the reaction mixture,

d) cycling, at least two times, the temperature of the reaction mixture between a first denaturation temperature, a first annealing temperature and a first extension temperature, the first annealing temperature being ~~similar to~~ approximately the same as or lower than the lowest T_m of the outer primer pair and higher than the highest T_m of the inner primer pair, and

e) cycling, at least two times, the temperature of the reaction mixture between a second denaturation temperature, a second annealing temperature and a second extension temperature, the second annealing temperature being similar to or lower than the lowest T_m of the inner primer pair,

~~f) optionally, analysing the product of step d) and/or step e) to detect the presence of the target nucleic acid sequence.~~

2. (Previously presented) The method according to claim 1, wherein the T_m of the pair of outer primers is 2-10 °C higher than the T_m of the pair of inner primers.

3. (Original) The method according to claim 1, wherein at least one primer of the outer primer pair comprises a T_m -increasing component.

4. (Currently amended) The method according to claim 1 ~~any of the claims 1-3~~, wherein both of the primers of the outer primer pair comprise a T_m -increasing component.

Appl. No. : **Unassigned**
Filed : **Herewith**

5. (Previously presented) The method according to claim 4, wherein the T_m-increasing component binds non-specifically to nucleic acids.

6. (Currently amended) The method according to claim 3 ~~any of claims 3-5~~, wherein the T_m-increasing component comprises one or more moieties selected from the group consisting of a modified nucleotide and a minor groove binding agent.

7. (Previously presented) The method according to claim 6, wherein the modified nucleotide is a peptide nucleic acid (PNA) or a locked nucleic acid (LNA).

8. (Currently amended) The method according to claim 3 ~~any of claims 3-7~~, wherein the T_m-increasing component increases the T_m of the primer with at least 1°C relative to the T_m of the same primer not comprising the T_m-increasing component.

9. (Currently amended) The method according to claim 1 ~~any of the preceding claims~~, wherein the second denaturation temperature is at least 1°C lower than the first denaturation temperature.

10. (Currently amended) A method for detection of *Bacillus anthracis*, the method comprising detecting a target nucleic acid sequence according to the method of claim 1-9, the target nucleic acid sequence being specific for the pXO1 or pXO2 plasmid of *Bacillus anthracis*, wherein the pair of outer primers and the pair of inner primers are selected from the pXO1 or pXO2 plasmid of *Bacillus anthracis*.

11. (Currently amended) The method according to claim 10, wherein the pair of outer primers and the pair of inner primers are selected so as to amplify a target nucleic acid sequence related to a gene selected from the group of *B. anthracis* genes consisting of capA gene, the capB gene, the capC gene, the lef gene.

12. (Currently amended) The method according to claim 10 ~~or 11~~, wherein target nucleic acid sequence is related to the capA gene and

Appl. No. : **Unassigned**
Filed : **Herewith**

- a primer of the pair of outer primers comprises a nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 2, a homologous sequence thereof, and a complementary sequence thereof, and
- a primer of the pair of inner primers comprises a nucleic acid sequence selected from the group of SEQ ID NO: 3, SEQ ID NO: 4, a homologous sequence thereof, and a complementary sequence thereof.

13. (Currently amended) The method according to claim 10 ~~any of the claims 10-12~~, wherein target nucleic acid sequence is related to the capA gene and the pair of outer primers comprises SEQ ID NOs: 1 and 2 and/or the pair of inner primers comprises SEQ ID NOs: 3 and 4.

14. (Previously presented) A kit comprising a pair of outer primers and a pair of inner primer, the melting temperature (T_m) of the pair of outer primers being higher than the T_m of the pair of inner primers.

15. (Previously presented) The kit according to claim 14, wherein the T_m of the pair of outer primers is 2-10 °C higher than the T_m of the pair of inner primers.

16. (Currently amended) The kit according to claim 14 ~~or 15~~, wherein at least one primer of the outer primer pair comprises a T_m -increasing component.

17. (Previously presented) The kit according to claim 16, wherein both of the primers of the outer primer pair comprises a T_m -increasing component.

18. (Currently amended) The kit according to claim 16 ~~any of the claims 16-17~~, wherein the T_m -increasing component binds non-specifically to nucleic acids.

19. (Currently amended) The kit according to claim 16 ~~any of the claims 16-18~~, wherein the T_m -increasing component comprises one or more moieties selected from the group consisting of a modified nucleotide and a minor groove binding protein.

Appl. No. : Unassigned
Filed : Herewith

20. (Previously presented) The kit according to claim 19, wherein the modified nucleotide is a peptide nucleic acid (PNA) or a locked nucleic acid (LNA).

21. (Currently amended) The kit according to claim 16 ~~any of the claims 16-20~~, wherein the T_m-increasing component increases the T_m of the primer with at least 1°C relative to the T_m of the same primer not comprising the T_m-increasing component.

22. (Currently amended) A kit according to claim 14 ~~any of the claims 14-21~~ for detection of *Bacillus anthracis*, the kit comprising a pair of outer primers and a pair of inner primer, the melting temperature (T_m) of the pair of outer primers being higher than the T_m of the pair of inner primers, wherein the pair of outer primers and the pair of inner primers are selected from the pXO1 or pXO2 plasmid of *Bacillus anthracis*.

23. (Previously presented) The kit of claim 22, wherein the pair of outer primers and the pair of inner primers are selected so as to amplify a target nucleic acid sequence within a gene selected from the group of *B. anthracis* genes consisting of capA gene, the Cap B gene, the Cap C gene, the lef gene.

24. (Currently amended) The kit according to claim 22 ~~or 23~~, wherein target nucleic acid sequence is related to the capA gene and

- a primer of the pair of outer primers comprises a nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 2, a homologous sequence thereof, and a complementary sequence thereof, and
- a primer of the pair of inner primers comprises a nucleic acid sequence selected from the group of SEQ ID NO: 3, SEQ ID NO: 4, a homologous sequence thereof, and a complementary sequence thereof.

25. (Currently amended) The kit according to claim 22 ~~any of the claims 22-24~~, wherein target nucleic acid sequence is related to the capA gene and the pair of outer primers comprises SEQ ID NOS: 1 and 2 and/or the pair of inner primers comprises SEQ ID NOS: 3 and 4.

Appl. No. : **Unassigned**
Filed : **Herewith**

26. (Previously presented) An analysis system for detection of a microorganism, the analysis system comprising a pair of outer primers and a pair of inner primer, the melting temperature (T_m) of the pair of outer primers being higher than the T_m of the pair of inner primers.

27. (Previously presented) The analysis according to claim 26, wherein the T_m of the pair of outer primers is 2-10 °C higher than the T_m of the pair of inner primers.

28. (Currently amended) The analysis system according to claim 26 ~~or 27~~, wherein at least one primer of the outer primer pair comprises a T_m -increasing component.

29. (Currently amended) The analysis system of claim 26 ~~any of the claims 26-28~~, wherein the analysis system is selected from the group consisting of a lateral flow device, a biochip, and a microarray.

30. (New) The method of claim 1, further comprising analyzing the product of step d) and/or step e) to detect the presence of the target nucleic acid sequence.